Comparative Study on Remineralizing Ability of Casein Phosphopeptide Amorphous Calcium Phosphate and β-Tricalcium Phosphate on Dental Erosion: An In vitro Atomic Force Microscope Study

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Abstract

Aim: To compare the remineralizing ability of between casein phosphopeptide amorphous calcium phosphate (CPP-ACP) and β-tricalcium phosphate (β-TCP) on dental erosion by soft drinks in human tooth enamel using atomic force microscope.

Materials and methods: 40 extracted human anterior teeth which were randomly divided into two groups: CPP-ACP paste (GC Tooth Mousse) and β-TCP (Clinpro™ Tooth Créam) paste demineralized in soft drink (Coca-Cola) for 2 min followed by remineralization by both pastes. Each sample was subjected to atomic force microscopy (AFM) for unexposed, demineralization and remineralization cycle for surface roughness determination.

Results and statistical analysis: Both experimental groups of samples shows remineralization that is reduction in surface roughness which was higher with β-TCP paste. Statistical analysis was performed using Student unpaired t-test and ANOVA along with Tukey’s post HOC analysis with p<0.01 in intra group comparison and not significant inter group comparison.

Conclusion: The β-TCP mineralizing paste is effective on preventing dental erosion.

Keywords: Remineralization; Demineralization; CPP-ACP; β-TCP; Atomic force microscope

Introduction

Dental erosion is a destructive process of losing tooth structure by acidic dissolution without bacterial involvement [1]. Continuous demineralization and remineralization takes place in oral environment. If this equilibrium disrupts, demineralization progresses deteriorating further tooth surfaces. This destructive process leads hypomineralized tooth surface to an irreversible loss and softening, thereby making it susceptible to fracture [2].

Caries lesion formation occurs by partial dissolution of hydroxyapatite of teeth. Erosion is the complete dissolution of hydroxyapatite layer by layer. Erosion may be of intrinsic, extrinsic and medicinal factors, occupational exposure or lifestyle origin. Frequent consumption and availability of acidic beverages, carbonated drinks, fruit juices, wine and sports drinks causes a drop in plaque pH and increase the prevalence of dental erosion as an extrinsic factor.

The process of restoring lost mineral ions to the tooth structure and strengthening the lattice work is known as remineralization. Calcium and Phosphate are the main minerals that get lost during demineralization. It can be stopped by creating an environment conducive for remineralization by non-invasive treatment by various calcium phosphate delivery systems. It is of three types: stabilized system containing CPP, unstabilized system containing ACP and crystalline system containing β-TCP.

Casein phosphopeptide (CPP) along with amorphous calcium phosphate (ACP) forms a protective layer and inhibits the demineralization. It is derived from bovine milk, casein, calcium and phosphates, β-tricalcium phosphate (β-TCP) is biocompatible, bioactive and a precursor of hydroxyapatite formation, manifests lattice defects that allow crystal modification.

Surface roughness assessment by atomic force microscope (AFM) is an important aspect as it affects the aesthetic properties as well as reflects the bacterial adhesion and plaque formation on the surface of tooth.

This study basically intends to compare the remineralizing potential of the dentifrices containing recently invented CPP-ACP and β-TCP on dental erosion produced by soft drinks on enamel using AFM.

Materials and Methods

A total of 40 extracted human anterior teeth were collected for the study. Teeth were cleaned by ultrasonic scaler to remove soft debris and inspected for cracks, hypoplasia or any white spot lesions. Further disinfection was carried out by immersing them in 5% sodium hypochlorite for 1 hour and storing in de-ionized water. The specimens were coronated and sliced longitudinally for the labial portion using diamond disc with water irrigation. The labial surface of each enamel specimen grounded using silicon carbide papers with consecutive 600, 1000 and 1200 grits under water irrigation for producing a flat surface.

The CPP-ACP paste used was GC Tooth mousse (GC Corp, Tokyo, Japan) and β-TCP paste was Clinpro™ Tooth Créam (3M ESPE, USA) (Figure 1).

The pH of soft drink was determined by pH meter.

All the samples were randomly divided into two groups. Group

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1 containing 20 samples of Casein-phosphopeptide along with amorphous calcium phosphate (CPP-ACP) dentifrice and Group 2 containing 20 samples of β-tricalcium phosphate (β-TCP) dentifrice.

Samples were subjected to erosion (demineralization cycle) in 6ml of soft drink (Coca-Cola) for 2 min stirred at constant speed of 120 rpms at four consecutive intervals of 6 h imitating meal time [1]. Remineralizing agents were applied at 0, 8, 16, 36 h intervals. GC tooth mousse is applied for 3 min and β-TCP for 2 min as per manufacturer’s instructions. AFM observations were made after each phase unexposed, demineralization and remineralization cycle.

AFM observations

Images were taken with Atomic Force Microscopy. For surface roughness assessment, Autoprobe CP 100 equipped with a piezoelectric scanner of tapping mode was used. The root mean square roughness, Rrms was obtained from different film areas with a resolution of 256 × 256 pixels. From the analysis surface roughened by surface erosion is determined (Figure 2).

Statistical analysis

Statistical analysis was performed using Student unpaired t-test and ANOVA along with Tukey’s post HOC analysis.

Result

The pH of soft drink was 3.04 ± 0.04 at room temperature.

The Rrms values of unexposed samples were in the range of 125.9 ± 61.4 and 170.77 ± 80.7 nm. After demineralization, all groups showed an increase in Rrms while on application of remineralizing agent a marked reduction in Rrms was present in Group 2 (β-TCP paste) with 158.03 ± 13.25 and 113.55 ± 7.77 nm followed by Group 1 (CPP-ACP) with 168.06 ± 13.14 and 114.81 ± 19.30

The change in Δ Rrms i.e. Δ UNEXP DEMIN, Δ UNEXP REMIN, Δ REMIN DEMIN was statistically highly significant in CPP-ACP than that in β-TCP Group (Figures 3-6).

The differences were statistically not significant upon intergroup comparison.

Discussion

AFM has been used in this present study to determine the surface roughness occurred after the erosion and to compare the protective effect of CPP-ACP and β-TCP on enamel. AFM gives a high resolution, high contrast structural image. Tapping mode (TM AFM) images were able to show net difference between exposed and unexposed enamel areas treated with demineralizing solutions [3]. AFM was first used by Hegedus et al. [4] to study effect of bleaching agent on enamel surface comparing with that of Scanning Electron Microscope (SEM). One of the advantages of AFM over SEM is that it can generate image on dehydrated enamel surface.

Enamel surface is highly mineralized and aprismatic as compared
to enamel subsurface which can be removed by polishing process. At the end of amelogenesis, this prismless enamel arises. It is found frequently on primary teeth but can also be found on the permanent teeth. This ‘prism free’ layer is worn due to mastication. In this study samples were made flat to standardize and to remove natural variations in surface enamel. Otherwise it may result in a different response to acid dissolution.

According to Lippart et al. even exposure to 13% sodium hypochlorite did not alter the nano mechanical properties or the demineralization effect on enamel even after 3 days [5]. Hence, disinfection in 5% sodium hypochlorite for 1 hour could not alter the enamel surface.

There is no clear relationship between phosphate ion and erosion but negative correlation present between calcium and erosion. Hence Coca-cola, the beverage with highest calcium level and low pH was chosen for in vitro study. Moreover, it is the mostly commonly consumed carbonated beverage. The beverage was replenished for every 2 min to ensure that it remain carbonated and reduce the buffering effect from ions dissolved from enamel surface.

There are three calcium phosphate delivery systems namely crystalline (β-TCP), unstabilized (ACP) and stabilized (CPP) systems. Different sequential crystal phases can be produced- brushite, octacalcium phosphate, hydroxyapatite, fluoroapatite. Fluoroapatite is the most acid resistant phase. Solubility increases from brushite to fluoroapatite.

β-TCP is precursor of hydroxyapatite formation. It is bio-active, biocompatible and allows crystal modification by manifesting lattice defects. β-TCP in combination with sodium lauryl sulfate is used so that calcium oxide does not react with fluoride ion, making it ineffective. This protective barrier breaks down when β-TCP comes in contact with tooth surface moistened by saliva, making calcium, phosphate and fluoride ions available to teeth.

Many studies describe the beneficiary effects of CPP-ACP. It increases the calcium binding sites, hence decreasing the calcium
diffusion constant [1]. It layers the interprism cavities and prisms for a long time. Thus, preventing teeth surfaces from subsequent acid attacks. CPP stabilizes ACP in metastable stage. Critical size of ACP required for nucleation and phase transformation is prevented by CPP. In dental plaque, CPP is to localize ACP and buffers free calcium and phosphate activities and maintain a super saturated state, thus, enhancing remineralization and decreasing demineralization [6,7].

This study provides a clinical relevance on the importance of minimal invasive treatment on incipient carious lesion by remineralization.

**Conclusion**

Within the limitations of the study it has been concluded that all the samples showed highly significant increase in surface roughness after demineralization and a significant reduction in surface roughness is seen in all samples after remineralizing by both the remineralizing agents. Greater remineralization was present in samples of β-TCP paste than CPP-ACP but was not statistically significant.

**References**